Syracuse Univ.

INFORMAL SEMI-ANNUAL REPORT ON RESEARCH GRANT NASA - NsG-693

"Studies on trace elements in the sporulation of bacteria and the germination of bacterial spores"

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I. Summary of Progress and Proposed Research

The cryptic manner in which the bacterial endospore is transformed into a vegetative cell has been the subject of intense interest in recent years in an attempt to elucidate intracellular differentiation at a basic level and to understand better the phenomenon of dormancy. The manifestations of this transformation are seen in three distinct sequential processes: activation, the conditioning of the spore to germinate under appropriate conditions; germination, the loss of the characteristics of the spore; and outgrowth, the formation of a new vegetative cell.

This grant has been concerned with the irreversible process of germination in an attempt to clarify the role of ions. The primary role of both micro- and macronutrient elements in cell physiology can be explained as active groups of various enzymes, and important in this biochemical function, are the physiological modes of action of ions, both specific and non-specific. These functions, such as ion exchange, ion antagonism chelation, permeation and maintenance of structural components have been shown recently to affect the process of germination.

Germination of Bacillus megaterium spores was examined from several different aspects with regard to ionic germination. First, a chemically pure and defined system was established and the effects of twenty one different cations, added to the germination system in trace amounts, were noted. Results indicated that molar ionic effects, in either a potassium phosphate buffered system or a sodium chloride unbuffered system, supplemented with the physiological germinants, L-alanine and inosine, were solely caused by these monovalent cations, and that any extraneous heavy metal or alkaline earth metal contamination displayed either inert or inhibitory activity in the germination process. The validity of the purification procedures was confirmed by the employment of atomic absorption spectrophotometric analysis techniques.

Further studies showed that spores raised synchronously by means of the techniques described in an earlier report exhibited a faster rate of germination than corresponding asynchronously raised spores. Using this system, distinct changes in the germinative ability of the spore have been observed which heretofore may have been masked. Spores raised synchronously and deficient in the cation, manganese, would not germinate under physiological or ionic inducive conditions. These changes in germinative ability may help us to understand a part of the "cryptobiotic" nature of the dormant spore and to identify the underlying factors in the spore's ability to germinate and alter its basic structure. These studies will be continued.

Another aspect of the work on this grant has been the discovery of temperature induced sporulation mutants in Bacillus subtilis. Spores of B. subtilis strain Marburg were obtained from cells grown on defined medium, collected into pellets by centrifugation of 1 ml of culture, and dried. These pellets contained 10^8 to 10^9 spores. After prolonged heating in vacuum at relatively high temperature the pellets were resuspended in medium salts and plated on modified potato dextrose agar (PDA). This procedure resulted in the appearance of considerable numbers of mutants while only a slight kill was observed. Many of these mutants were oligosporogenic or asporogenic, and a few were auxotrophic. The auxotrophic mutations did not seem to be related to the sporulation mutations. Best results were obtained

when a heating period of 10 to 12 hours, in vacuum, at temperatures near 100°C were used. Under these conditions less than one log kill was detected by plate counts, while 5 to 10% of the survivors were mutants. Selection was clearly eliminated. Mutants were detected primarily by observed changes in colonial morphology on PDA; and by changes in pigment production. Once collected they were examined for amino acid auxotrophy, acriflavin resistance, and sporulation ability as determined by microscopic examination and heat shock tests. Results show that acriflavin resistance and sporulation mutations appear to be independent events, but that both occur at significantly higher rates than auxotrophic mutations. It is also known that just acriflavin, in low concentrations on PDA plates, will give sporulation mutations and acriflavin resistance mutants at much higher frequencies than auxotrophic mutation. This evidence suggests that the process of sporulation may involve a cytoplasmic element. This element may be removed or damaged by high temperature or acriflavin under conditions which do not affect the main genome to a comparable extent.

While these studies support the hypothesis of episomic involvement as genetic determinants for sporulation previously presented from this laboratory (Rogolsky and Slepecky, BBRC 16:204-208 (1964), they are germane to the problem of dormancy and elimination of spores by high temperatures, a problem of much concern to NASA in their planetary quarantine program. For example, we are concerned not only with the elimination of the sporulation capacity but also, whether such high temperatures may induce mutants with increased heat resistance, a heretofore unstudied possibility.

II. Publications during the Period of the Report

Rogolsky, M. and Slepecky, R. A. Induction of Asporogeny in *Bacillus subtilis* with Acriflavine. Bact. Proc. 1965:36 (an abstract).

Gillis, J. R., Rosas del Valle, M., Vinter, V. and Slepecky, R.A. Sporulation studies in a synchronous culture of *Bacillus megaterium*. Bact. Proc. 1965:37 (an abstract).

Gruft, H., Buckman, J. and Slepecky, R. A. Amino acid replacement of the manganese sporulation requirement of *Bacillus megaterium* in a synchronous system. Bact. Proc. 1965:37 (an abstract).

Slepecky, R. A. Spores III. ASM News 31:66-67 (1965) (A book review).

Vinter, V. and Slepecky, R. A. Direct transition of outgrowing bacterial spores to new sporangia without intermediate cell division. J. Bacteriol. 90:803-807 (1965).

Slepecky, R. A. The use of combined sonication-germicide treatment in surgical instrument cleaning. Hospital Topics (in press).

Remsen, C. C., Lundgren, D. G. and Slepecky, R. A. Inhibition of spore septum and forespore membrane development in *Bacillus cereus* by beta-phenethyl alcohol. J. Bacteriol. (in press, due in January 1966 issue).

III. List of Personnel Engaged in the Project during the Period of the Report

Dr. Ralph A. Slepecky, Principal Investigator
Miss Zita Celkis, Technician
Mr. Jeffrey Buckman, Research Assistant
Mr. Howard Gruft, Graduate Student
Mr. Jere Northrop, Research Fellow
Mr. John Gillis, Graduate Student (present address: Missile and Space Division, General Electric Co., P.O. Box 8555, Philadelphia, Pa.).